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OCCURRENCE OF PARALLEL SERIES OF ASSOCIATED PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERS IN DIVERSE GROUPS OF MOSQUITOES AND OTHER INSECTS

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INTRODUCTION

SEVERAL characters which are useful for the differentiation of species and subspecies of insects recur in association in widely separated taxonomic groups. Combinations of these characters are present in insects as widely separated as the three major groups of mosquitoes (*Anopheles, Culex,* and *Aedes*), flies of the genus *Drosophila,* and butterflies of the genera *Colias* and *Melitaea.* Knowledge of similar associations between characters in other insect groups is limited only by the paucity of information. Some similar parallel associations are known to be present in other classes of animals.

Most of the physiological characters that are known to be associated are related in some way to the rate of body metabolism. Most of the morphological characters concern differences in size or in proportional development of the body parts. Color characteristics are also involved.

It is the purpose of this paper to illustrate these parallel associations in diverse groups of insects and to discuss the possible mechanisms by which they may have originated.

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KINDS OF CHARACTERS

Combinations of the following characters are associated in a particular order among the various forms of the groups discussed. Owing to differences of opinion in the literature regarding the taxonomic standing of many of these forms, the terms ''species,'' ''subspecies,'' and ''race'' have been avoided in this paper. In their place, the term ''form'' has been used.

A. Development rate. Insect development can be measured in definite units corresponding to the molting periods. In the higher insects there is no growth after the adult stage is reached. It is possible, therefore, to determine the time elapsed for the complete development of the organism or of any part of this life cycle.

B. Activity. In the adult insect it is not possible to measure growth as a characteristic, but it is possible to measure general activity as a quantitative character. This is usually accomplished under controlled laboratory conditions.

C. Mating habits. Some insects mate more readily in small enclosed spaces than do others. Behavior of the former class has been denoted by the term ''stenogamy'' and of the latter class by ''eurygamy.''

D. Ecological distribution. Preference for certain types of environments may be considered a characteristic of an organism in the same way as is any other physiological trait. The environments may be compared as north or south, high or low, hot or cold, dry or humid.

E. Coloration. Usually, only the major color of the insect body is mentioned, that is, the body may be dark or light. The coloration is primarily due to a browning of and the deposition of melanin pigment in the chitin. In the case of pigments of a different chemical origin, as in the Lepidoptera, specific mention is made of the fact.

F. Larval food tolerance. Some mosquito larvae are better able to live in heavy infusions of micro-organisms than are others. This is mentioned as the larval tolerance to strong infusion.

G. Larval tolerance to salt. Some mosquito larvae are better able to exist in water containing salt than are others. This is mentioned as the larval tolerance to salt solution.

H. Adult feeding preference. Some mosquito adults prefer a blood meal from man rather than from other animals, or will attack man more often than do others.

I. Morphological characteristics. The number of hairs emerging from a given locus on the body and the length of the hairs are variable and significant. The relative lengths of parts of the body to one another are also variable and significant. These differences in growth or in body proportions have been mentioned wherever such data are available.

THE ASSOCIATED CHARACTERS IN MOSQUITOES

Culex pipiens

Culex pipiens is one of the common mosquitoes of Europe. The names *molestus* and *pipiens* are used for two forms of this mosquito which are differentiated by certain combinations of physiological and morphological characteristics. These traits are listed in Table I and apply especially to the mosquito strains studied and reviewed by Jobling (1938), Tate and Vincent (1936), and Marshall (1944).

The chitin color of specimens of *Culex molestus* is a ''light reddish almost rust color with golden down,'' whereas that of specimens of *pipiens* is a ''dark cigar brown'' with the tarsal extremities almost black. *Culex pipiens* is, therefore, darker than is *molestus*. The white knee spots of *pipiens,* noted by Marshall (1944), are a trend toward a paler color. This is, however, a much less significant character than is general body color.

In Europe, the darker form, *pipiens,* exists in the north, the lighter, *molestus,* occurs in the south; there is considerable overlapping in ranges.

C. molestus is a mosquito which, especially in the north, inhabits human dwellings. It is dispersed rapidly by man. In the north *C. pipiens* occupies the natural habitats.

Although *molestus* does not require a blood meal for the development of eggs, it prefers to bite man rather than birds when it does take a blood meal. This fact combined with its domestic breeding habits makes *molestus* a nuisance to mankind. *C. pipiens* prefers birds to man.

C. molestus is able to breed in extremely small enclosed spaces; this permits its engagement in its domestic habits, in which mating often occurs in small containers. The absence of a diapause in this variety shows that it is of a southern or warm climate adaptation. The evidence suggests that it is unable to overwinter under natural conditions in any northern European locality. In central Europe, it overwinters by breeding continuously in relatively warm places such as cellars, cesspits, and animal houses.

Tests on the comparative developing rates of these races made by Jobling (1938) are inconclusive, probably because of the wide temperature fluctuations during breeding of the mosquitoes (as much as 9.5° C. in most of the laboratory experiments). The data do show, however, that specimens of *pipiens* may be able to survive better or for a longer period of time below a mean of 13.5° C. than do those of *molestus*. Larval survival in salt solutions at varying concentrations is greater in specimens of *molestus* than in those of *pipiens;* likewise, survival of the larvae in a more concentrated larval infusion is greater in *molestus* than in *pipiens* (Jobling, 1938).

TABLE I

PHYSIOLOGICAL AND MORPHOLOGICAL DIFFERENCES BETWEEN THE TWO PRIMARY EUROPEAN FORMS OF *Culex pipiens*

Character	*molestus*	*pipiens*
A. Larval development	Poor survival at low temperatures	Better survival at low temperatures
B_1. Activity at low temperatures (adult)	Poor	Good
B_2. Diapause	Absent	Present
C. Mating habits (comparative size of area necessary for copulation)	Small (stenogamy)	Large (eurygamy)
D. Ecological distribution	Southern (warmer, dryer)	Northern (cooler, wetter)
E. Color (adult)	Light	Dark
a. Dark spots on abdomen	No	Yes
b. Chitin color	Light	Dark
c. White spots on legs	No	Yes
F. Tolerance to strong infusion	Good	Poor
G. Tolerance to salt solution	Good	Poor
H. Food requirements	*a.* Prefers man *b.* Needs no blood for egg development (autogeny)	*a.* Prefers birds *b.* Blood is necessary for egg development (anautogeny)
I. Morphological characteristics:		
a. Number of branches of hairs	Fewer	More
b. Hair length on larvae	Shorter	Longer
c. Comparative length: palpi to proboscis	Palpi shortest	Palpi longest
d. Siphonal index	Smaller	Longer
e. Branches in the siphonal tufts of the larvae (seem to be longer in *pipiens,* however)	More	Less

C. molestus and *pipiens* are not isolated by any known genetic mechanism. When growing in similar places in their natural habitats, cross-breeding and the production of hybrid intermediates occur. Tate and Vincent (1936) summarized cross mating experiments between these two races. Characteristic of these forms was (1) the necessity for a blood meal in order to lay eggs and (2) the ability to mate in a very small space. Neither of these characters is necessarily unit-controlled in heredity; that is, it is possible that a complete series of intergradations occur, the two extreme halves of which have been fitted, more or less, into the classifications as given above. In the F generation, if this is the case, all individuals should be intermediate between the parents. Owing to the nature of the technique it was not possible to discover an intermediate condition.

Tate and Vincent (1936) stated that "in the cross-bred adults stenogamy (mating in small space) appears in most of the progeny, if not all, even in the F_1 generation, whilst autogeny (no blood necessary for the production of eggs) is exhibited by only a small proportion of the progeny of the first three generations." Individuals of *molestus* will mate in any cage large enough to move around in. In the particular experiment mentioned, the egg rafts produced by the F_1 individuals were very small. Specimens of *C. pipiens* produced large rafts and those of *molestus* produced small ones after a blood meal; only *molestus* produces eggs without a blood meal. Perhaps an intermediate condition in this character is suggested in the latter experiment. It is possible, likewise, that the mating behavior would also have been intermediate were a different technique used for testing the character.

The data on nine generations inbred from two intermediate F_1 egg rafts suggest a strong selection in the first two generations for those individuals which mated in a small space and laid eggs without a blood meal. This selection probably continued for the duration of the interbreeding, but showed no effects after the fourth or fifth generations, at which time there was developed a selected strain comparing well with any pure line of *molestus*. This would be expected under the circumstances, in which the selection began with genetically F_1 *pipiens* × *molestus* material and followed the expectations of multiple factor inheritance. Normal laboratory strains remain pure lines of either *molestus* or *pipiens* despite the constant selection in favor of *molestus,* owing to the fact that only a limited genetic variability is available to be selected for in the original strain and selection would have no effect.

Forms having characteristics not identical with those of *molestus* nor *pipiens* recently have been found in Europe (Callot and Van Ty, 1943). These, however, may represent hybridization products of the other two forms.

Anopheles maculipennis

The *Anopheles maculipennis* forms have been of interest since their discovery by Roubaud (1921). There are at least seven forms distinguishable in Europe. These occupy greatly overlapping territories, but are distinct in adult and larval ecologic-niche preferences (Bates and Hackett, 1939). There are distinct morphological differences in the number and branching of the hairs of the larvae (De Buck, Schoute, and Swellengrebel, 1930; Bates, 1939*a*) or in the size of the float in the egg (Missiroli, *et al.,* 1933). The color and pattern of the eggs also serve to distinguish them (reviews, Missiroli, *et al.,* 1933; Missiroli, 1938; Hackett and Missiroli, 1938). It is possible, though not mentioned in the literature, that slight differences in darkness or lightness of chitin in the adults and the larvae exist that are correlated with the egg color characters. Differences exist in types of "mating dance," or rather in premating tactics. These seem to be primarily a matter of the amount of space required for copulation to occur (Bates, 1941). The races have varying larval tolerances for sea water (Bates, 1939*b*), and the adults have varying preferences for taking human or animal blood (Hackett and Missiroli, 1935; and see below). Within any given locality there is a greatly different seasonal distribution of the races (Bates, 1937; and Lewis, 1939). These are dependent upon the seasonal temperature changes.

Data of these sorts have been summarized in Table II, giving for each race a number corresponding to its position with respect to a given character, for example, the lightest race is number 1, the second lightest is 2. Where it has been impossible to derive the relevant data from the literature a question mark has been substituted for the number. Table III is a correlation, showing the number of times that a given race has been indicated by a given number. A clear correlation is shown for most of the characters.

TABLE II

PHYSIOLOGICAL AND MORPHOLOGICAL DIFFERENCES BETWEEN THE FORMS OF *Anopheles maculipennis* OF EUROPE

Degree of manifestation of each character except H and I_2 is indicated by the numbers from 1 to 6.

Character	*elutus (sacharovi)*	*labranchiae*	*atroparvus*	*maculipennis*	*messeae*	*subalpinus*	*melanoon*
C. Mating habit (needs small to large cage)	2	2	1	4	5	5	?
D. Ecological distribution (from south to north, low to high elevation, hot to cold part of year)	1	2	3	4	5	3	3
E. Egg color (from light to dark)	1	2	3	4	5	?	6
G. Tolerance to sea water (good to bad)	1	?	2	4	4	3	?
H. Adult feeding preference (prefers man = M, prefers animals = N)	M	M	M	N	N	N	?
I_1. Egg float size (from small to large)	1	2	3	4	5	?	6
I_2. Larval chaetotaxy (actual mean number of branches of antepalmate hairs are given)	31	10	11	13	15	22	24

With *Anopheles maculipennis,* as with *Culex,* these data illustrate that the characters are genetically correlated and seem to be controlled by a multiple factor (blending) inheritance mechanism. The F_1 hybrids are intermediate in mating habits. The larval hair-number characters (chaetotaxy) are also exactly intermediate in the F_1 hybrids (Bates, 1939*a*, 1941; but see below).

As with *Culex,* the lighter forms prefer to bite man. In *Anopheles,* these are *elutus* (*sacharovi*), *labranchiae,* and *atroparvus.* These three mosquitoes are the malaria vectors in the regions in which any of the races occur in the same locality. Bates and Hackett (1939) stated that this man, or nonman, preference terminology is unfortunate, since the former implies a special preference for man over all other animals and the latter a preference for animals over man. Rather than this, *typicus* (*maculipennis*), *messeae,* and *subalpinus* have a narrower range of hosts. All these mosquitoes prefer to bite cattle or rabbits when man is the alternative; the difference between the types seems to be that some are more general in feeding habits than others or that some respond to weaker stimuli than do others. Thus, *atroparvus* and *labranchiae* respond to the weaker stimuli produced by man, whereas the others would not. Nevertheless, Van Thiel (1939) stated that zoophilism *vs.* anthropophilism is a definite smell preference by the mosquitoes.

TABLE III

CORRELATION SHOWING THE NUMBER OF TIMES THAT A GIVEN FORM IS REPRESENTED BY A GIVEN NUMBER IN TABLE II

Form	Number of Times						
	1	2	3	4	5	6	Uncertain
elutus	4	1	......	......	......	......	0
labranchiae	......	4	......	......	......	......	1
atroparvus	1	1	3	......	......	......	0
maculipennis	......	......	......	5	......	......	0
subalpinus	......	......	2	......	1	......	2
messeae	......	......	......	1	4	......	0
melanoon	......	......	1	......	......	2	3

It was observed by Van Thiel (1926) that the form inhabiting the brackish-water areas in Holland had shorter wings (*atroparvus*) than did the fresh-water form (*messeae*).

Hackett and Missiroli (1935) reviewed the data on the structure of the spines on the male genitalia of the forms. According to their figures (from material of La Face), the darker forms, *melanoon* and *messeae,* have much thicker and heavier spines than do the lighter forms, *atroparvus* and *labranchiae,* whereas the intermediate type, *maculipennis,* itself is intermediate. Thus, details in male genitalia may be ecogenotypically as variable as any other end product of a complex development.

These authors provide illustrations of variation in structure of a hair of the second abdominal segment of the larvae. The hairs on the darker forms (*melanoon* and *messeae*) are greatly expanded and developed, in the lighter forms (*labranchiae* and *atroparvus*) they are short and stubby, whereas in the intermediate form (*maculipennis*) they are intermediate. These differences agree with those of the antepalmate hairs described below and the male genitalia spines described above.

Data on the branching of the antepalmate hairs of the various *maculipennis* forms have been summarized by Bates (1939*a*). These data do not agree completely with those obtained on the other characters, unless the forms are divided into subsections, as has been suggested by Hackett and Bates (1939), Bates (1940), and others, who place *sacharovi* as a specific unit separate from the *maculipennis* complex. These sections are: (1) *sacharovi* (*elutus*), (2) *subalpinus* and *melanoon,* (3) *labranchiae, atroparvus, maculipennis,* and *messeae.*

Within each of these divisions, the number of branches on the antepalmate hairs follows the same pattern of variation. Likewise, most exceptions to the rule as noted for the other characters are eliminated.

Hybridization of some *A. maculipennis* forms led to the determination that the F_1 hybrids are intermediate between the parents in the branching of the antepalmate hairs (Bates, 1939*c*). Hybrids tested were as follows (the numbers represent the hair number): *atroparvus* (11) × *subalpinus* (22) giving F_1 (16); and *atroparvus* (11) × *sacharovi* (31) giving F_1 (17).

In geographical and ecological distribution, each form of the group is distinct, but overlaps some one or more of the others. Despite this overlap, there seems to be little natural hybridization among them. The data of De Buck, Schoute, and Swellengrebel (1934) and Bates (1939*c*) suggest that intercrossing would be rather ineffective in transferring genes from one population to the other. Sterility of one sort or another seems to result from intercrossing; yet in some instances hybrid individuals are fertile and give rise to fertile progeny in backcrosses.

In distribution the darker forms are more northern and the lighter are more southern. Where the two occur in the same general locality, for instance in Albania, the darker are commoner at the higher and cooler elevations than are the lighter. There is a definite segregation of the races latitudinally and climatically, even though a tremendous overlap occurs (Lewis, 1939; Bates and Hackett, 1939). The distribution of *melanoon* and *subalpinus* seems less definitely known than that of the others and, on this account, their distributions are more difficult to correlate.

In the several parts of Albania, there is a great change in the frequencies of the forms at various times of the year (Lewis, 1939). At the sea-level localities, *subalpinus* and *maculipennis* (both intermediate in color) are most abundant in the spring; there is an increase of each in population as the season progresses. There is, however, an even greater increase of *sacharovi* (lightest colored). At an intermediate elevation in the mountains *maculipennis* is most abundant, but in the warmer part of the year the population increase of *subalpinus* and *sacharovi* is comparatively greater. At the locality of highest elevation, *maculipennis* is most common early in the season, but is largely superseded during the warmer season later by *subalpinus.*

Ecological segregation also exists with respect to the type of breeding place selected by a form. For example, in northern Europe *atroparvus* occurs along the coasts, usually in brackish water; in the south, however, it breeds in fresh water and at inland localities. *A. labranchiae* is confined to the coastal areas of the Mediterranean west of the Adriatic; this is the part having the warmer winter temperatures. In the southern parts of its range it breeds in all habitats; in the north, breeding is only in brackish water. *A. messeae* is a continental type, breeding only in fresh water and preferring situations where the winters are cold. *A. sacharovi* is the Near East form occurring in the Balkans and along the coast of Italy, in Albania only in the coastal marshes. In the hottest part of the summer, it ranges inland. *A. maculipennis* itself is the most widely distributed of all, but it is ecologically intermediate in its preferences. It gives way to *atroparvus, sacharovi,* and *labranchiae* in the south and to *messeae* in the north. In Albania, during the warm season, it is replaced by *sacharovi* at the lower elevations and by *subalpinus* at the higher elevations.

The evidence suggests that the races which seem to prefer brackish or salty water in any region may not actually prefer it, but are merely able to tolerate it. They may live in brackish water within an area because it may be the only place available in the vicinity not already occupied by another form better suited for competition there. For example, in the northern parts of the distributions of each of the southern forms, *atroparvus, labranchiae,* and *sacharovi,* the larvae breed in salt water. In the southern parts of their ranges in the absence of the more northern races, they also occupy fresh water. The range of each of these forms is different. Thus, three factors may regulate the coastal, brackish-water distributions of these southern mosquitoes in the north: (1) the colder winter temperatures inland, (2) the competition inland with the northern forms, and (3) the better tolerance to salt water of the lighter or more southern forms.

In sexual behavior, the forms align themselves in order corresponding to the color types. These are shown in Table II (from De Buck, Schoute, and Swellengrebel, 1930; Corradetti, 1937; Bates and Hackett, 1939; Bates, 1941). The observation in *Haemagogus* (Hovanitz, 1946*a*) that this behavior is of degree only and therefore is likely regulated by a complex hereditary mechanism is illustrated by the results of a cross between a form that will mate in a cage of any dimension (*atroparvus*) and one that will mate only in a very large cage (*typicus* or *maculipennis*). The hybrid individuals swarm (the preliminary to mating) in the same size cage as the form *labranchiae*. *A. labranchiae* is intermediate between *atroparvus* and *maculipennis.*

Haemagogus

Haemagogus mosquitoes are closely related to the *Aedes* group. Eight forms are present in the northwestern part of South America. Most of these have different geographical distributions. In some places as many as three forms occur in the same forest patch, others seem to be replaced geographically by another form (Kumm, *et al.*, 1946).

Judging from geography alone, one may divide these *Haemagogus* forms into the following groups: (1) *falco,* (2) *equinus, andinus,* (3) *splendens, lucifer, boshelli,* and possibly *chalcospilans,* (4) *anastasionis.* By qualitative morphology, these same groups are apparent (Table IV).

H. anastasionis, chalcospilans, equinus, and *lucifer* occur in various parts of Central America, but not south of Colombia in the same form. *H. andinus* occurs at higher elevations in a cooler climate than do the other species. *H. lucifer, splendens,* and *boshelli* occupy contiguous geographical areas without overlapping. *H. splendens* occupies all the hot area in northeastern Colombia and adjacent Venezuela, which is characterized by a very long dry season; *lucifer* occupies the more central parts of Colombia; *boshelli* occupies the rainy coastal area of the Chocó, where no dry season seems to exist.

TABLE IV
COMPARISON OF SOME QUALITATIVE CHARACTERS OF COLOMBIAN *Haemagogus* MOSQUITOES

Character	*splendens, lucifer, boshelli, chalcospilans*	*equinus*	*andinus*	*anastasionis*	*falco*
Antennae of male heavily or lightly plumose	Light	Heavy	Heavy	Heavy	Heavy
Palpi length	Short	Long	Short	Short	Short
Tarsal claws of female toothed	No	Yes	Yes	Yes	Yes
Postnotal setae	Present	Absent	Absent	Absent	Absent
Second marginal cell on wing longer or shorter than petiole	Longer	Shorter	Shorter	Shorter	Shorter
Comb scales on larvae	Patched	In line	In line	In line	In line
White spots on femora	Absent	Present	Present	Absent	Absent
White scales on thoracic lobes	Absent	Present	Absent	Present	Present
Sternopleural setae of larvae	Absent	Present	Present	Absent	Absent

H. falco reaches its most northern limit in Panamá. Geographical differences are apparent within this group: the populations of eastern Colombia are different from those of the western area (Table V). *H. anastasionis* has a sporadic distribution in Colombia. Quantitative morphological and physiological characters separating these *Haemagogus* are given in Table V.

For the physiological characters, numbers are given to each species, as in *Anopheles,* to represent the relative position with respect to the other races in the quantitative characterization of the racial features. In the group *splendens-lucifer-boshelli, splendens* is at one extreme, *boshelli* at the other, and *lucifer* is in the intermediate position. Each character corresponds to a similar one in the *Anopheles* group. Of the other *Haemagogus, H. equinus* is the second lightest in color and generally has second place in the table. *H. andinus* is the darkest, with the possible exception of *boshelli,* and is generally in the last place. In the *equinus-andinus* group, *equinus* takes precedence over *andinus*. *Haemagogus anastasionis* is an intermediate entity, though it is unique. *H. falco* also stands apparently unique, but it is darker than is *anastasionis*.

As in *Anopheles* there seems to be a constant seasonal change in the frequencies of the forms in the same locality as well as regularities in the spacial distribution. For example, at a place called Volcanes, where *lucifer*, *equinus*, and *falco* occur together in the same forest, Boshell recorded for May and June, 1943, all the males as *lucifer* (Kumm, *et al.*, 1946). In October, 1943, during a dry period, 54.8 per cent of the males were *equinus* and 45.2 per cent were *lucifer*. No males of *falco* had to that date been obtained in the wild. In the same May and June above, 88.3 per cent of the females caught were *falco;* in October and November, most were *equinus*.

TABLE V

COMPARATIVE RATINGS OF PHYSIOLOGICAL AND MORPHOLOGICAL DIFFERENCES BETWEEN THE RACES AND SPECIES OF COLOMBIAN *Haemagogus* MOSQUITOES

	splendens	*lucifer*	*boshelli*	*equinus*	*andinus*	*anastasionis*	*falco*
A. Development rate (fastest to slowest)	2	4	5	1	?	?	3
B. Activity of adults (25–28° C.)	1	3	3	2	4	?	5
C. Ease of mating in cages (small to large)	1	3	3	2	4	?	5
D. Ecological distribution (hot-arid to cool-humid)	1	4	6	2	7	3	5
E. Adult coloration (light to dark)	1	5	6	2	7	3	4
F. Larval ability to withstand strong infusion (strong to weak)	1	2	3	1	3	?	2
I. Morphological characteristics: (mean numbers)							
Upper posterior pair of head hairs	1	1	1	1	2	1	1
Lower anterior pair of dorsal head hairs	2	2	2	1	1	1	1
Hairs in ante-antennal hair tuft	3	5	5	3	8	3	3
Scales in comb	18	24	34	8	10	8	7
Scales in pecten	16	13	14	12	17	15	10*
Hairs in lateral tuft on anal segment	2	3	2	2	5	5	2*
Ratio: siphon length to width	1.5	1.5	2	2	3	2	1.5
Scales in pecten tuft	3	3	4	2	3	3	2*
Setae tufts on side piece, male genitalia	0	1	3	?	?	?	?
Setae on venter of male adult	0	?	?	Few	Many	?	?

* These scales and hairs are longer in *falco* than in *equinus;* in the case of *falco*, there are four hairs instead of two in material from Leticia in far southeastern Colombia; likewise in the case of the ante-antennal tuft there are three hairs in the material from east of the Cordillera Oriental and two in that from west of there.

In another locality where the rainy season catch is *andinus*, during the dry season only specimens of *anastasionis* were obtained. The trend seems to be toward the darker form during the rainy, cloudy, or cooler part of the year and toward the lighter forms during the dry, sunny, and warm part of the year. This is similar to the trend noted for *Anopheles maculipennis.*

Anopheles albitarsus

A species or variety of *Anopheles albitarsus,* characterized by its domestic habits and by its fondness for taking human blood, exists throughout the central lowlands of Brazil in the vicinity of Rio de Janeiro. It is a notorious malaria vector (A. L. Ayroza-Galvão, personal communication). This race has been given the name *Anopheles albitarsus domesticus.* At the higher elevations at São Paulo, Brazil, there exists another variety (*A. albitarsus albitarsus*) which is not an effective vector of malaria; it lacks domestic habits and feeds rarely on human blood. This race also lives throughout the temperate parts of Argentina to the south. It apparently prefers cool living conditions in contrast to *domesticus,* which occupies the tropical lowlands to the north. In body characters the two races are distinguished by the extent of melanization of the tarsus; in specimens of *domesticus* the second hind tarsus is only about 50 per cent melanic, whereas in those of *albitarsus* (typical form) the corresponding structure is about 70–95 per cent melanic. Therefore, the character correlations compare with those in other mosquitoes.

THE ASSOCIATIONS BETWEEN CHARACTERS IN OTHER INSECTS

Kühn and von Engelhardt (1937) have shown that a genetic mutant of a moth in which the wings are suffused with melanin pigment has greater viability under conditions of lower temperature and increased humidity than has the same moth without the mutant effects. It also has a slower development rate. This is a pleiotropic effect of a single gene.

For many insects the data which point toward a correlation between body color and ecological living preference, especially in the Lepidoptera, have been received earlier (Hovanitz, 1941). The correlations correspond to those in the mosquitoes.

The genus *Drosophila* has similar correlations. Table VI shows the correlation between the development rate of twenty-two species of Japanese *Drosophila* and the amount of darkness which these species exhibit in their body color. Darkness is associated with slow development rate and lightness with rapid development rate. Correlations with the nature of the environment as judged by northern versus southern distributions also have some significance. The average length of development for all species listed by Kikkawa and Peng (1938) as Arctic or temperate in geographical distribution is 14.6 days (thirteen species); for those with a tropical distribution, the average is 10.5 days (seven species). The growth rates were tested in the laboratory at a comparable temperature of 26° C.

The comparative rates of development between some closely related *Drosophila* species or races which differ in chitin color may be illustrated by *D. pseudoobscura* (race A), *D. pseudoobscura* (race B or *persimilis*), and *D. miranda.* These species or races occupy contiguous or overlapping geographical regions from south to north in the order given. *D. miranda* is the darkest in color, whereas race A of *pseudoobscura* is the lightest; races A and B are not obviously different in this respect. Under the same conditions, *D. miranda* develops the slowest of the three. *D. pseudoobscura* race B (*persimilis*) is second, and race A develops the fastest (Poulson, 1934; Dobzhansky and Epling, 1944).

These differential developing rates, colors, and ecological preferences are associated with some other physiological characteristics. At 25° C., race A deposits more eggs than race B; at 19° C., both races are about equal in fecundity; and at 14° C., race B is superior to race A (Dobzhansky, 1935). The life expectancy of race A in the absence of food is greater than that of race B (Lilleland, 1938). This may be an indication of a greater resistance to desiccation in race A than in race B. Apparently, there is a slightly different rate of oxygen consumption in the two forms (Dobzhansky and Poulson, 1935). The frequency of wing beats per unit time in flight has been found to be greater in race A than in race B (Reed, Williams, and Chadwick, 1942). This may be an index of activity of the adults. Mather and Dobzhansky (1939) have found that the number of sex comb teeth is slightly greater in A than in B and that the wings of B are slightly larger than those of A. The wings of specimens of *miranda* and the body size in general are larger than in either race of *pseudoobscura;* also the teeth in the sex combs are more numerous than in *pseudoobscura.* These characteristics, except for sex comb number in races A and B, are parallel to corresponding characters in the mosquito races. These data are summarized in Table VII.

TABLE VI

Correlation Between Development Rate and Coloration of Adults in Japanese *Drosophila*

Development data from Kikkawa and Peng (1938); classification into arbitrary color classes by A. H. Sturtevant (personal communication). Arbitrary scale of pigmentation: lightest = 1, darkest = 5.

Development Days at 26° C.	1	2	3	4	5
10	2	3	0	0	0
12	2	5	0	1	0
14	1	1	1	1	0
15	0	0	0	1	0
16	0	0	0	1	0
18	0	0	0	1	0
20	0	0	0	1	0
25	0	0	0	0	1

In the races and color forms of *Colias* butterflies in North America, correlations of the same type between genetic characters of growth and form, color, and geographical distribution are present. The correlations between color and form hold whether races (or close species) are considered (Hovanitz, 1944*ab*) or whether a single genetic mutant within a race is considered (Hovanitz, 1944*c*, 1945*a*). Color, ecological distribution, laboratory environmental preferences, and development rate so far as is known are similarly associated. With *Colias*, it was possible to illustrate by experimental manipulation of the environmental conditions how the resulting change in metabolic rate affects directly the morphological and color characteristics of the organism (Hovanitz, 1946*b*). As in the mosquitoes, the race with the higher metabolic rate and southern-type characteristics has been better enabled to become a pest to mankind (Hovanitz, 1944*d*).

TABLE VII

COMPARATIVE PHYSIOLOGICAL AND MORPHOLOGICAL DATA ON THE *Drosophila pseudoobscura* GROUP

	pseudoobscura A	*pseudoobscura B* (*persimilis*)	*miranda*
A. Development rate (fastest to slowest)	1	2	3
B_1. General activity	High	High	Low
B_2. Frequency of wing beats (high to low)	1	2	?
B_3. O_2 consumption (probable) (higher to lower)	1	2	?
D_1. Ecological distribution (warm-dry to cool-wet)	1	2	3
D_2. Fecundity best at temperatures	High	Low	?
D_3. Life expectancy without food (good to bad)	1	2	?
E. Color (light to dark)	1	1	2
I_1. Number of teeth in sex combs (low to high)	2	1	3
I_2. Wing size (small to large) ♂	2	1	3
I_3. Tibia length	2	1	?

The races of *Melitaea chalcedona* exhibit a similar array of characteristics (Hovanitz, 1942, and unpublished observations). The darkest races inhabit ecological areas having cooler and wetter environmental conditions; the lightest races inhabit ecological areas having hotter and dryer environmental conditions. Laboratory observations indicate that the light races will mate more easily in small cages than will the dark. Likewise, the developmental rate of the lighter is more rapid than that of the darker.

DISCUSSION

The preceding examples illustrate the occurrence of a parallel series of associated physiological and morphological characters in several different species groups of mosquitoes and other insects. The associated characters are various qualities of (A) development rate, (B) activity of adults, (C) mating habits, (D) ecological distributions, (E) coloration, (F) larval food, (G) salt tolerances, (H) adult feeding preference, and (I) morphological characteristics.

In each instance where information is available (Table VIII), that is, for *Culex pipiens* forms, *Haemagogus* forms, *Drosophila* species, *Drosophila pseudoobscura* forms, *Colias* forms, and other Lepidoptera, there is a range in variation of the species from those with a slow to those with a fast rate of development. These differences in the rates of development are correlated with the variation of certain other characteristics. For example (Table VIII), some forms of *Culex pipiens, Haemagogus, Drosophila pseudoobscura, Colias,* and other Lepidoptera have, in association with the rapid development rate, a greater adult activity. In *Culex* and *Colias* the existence of a diapause is considered an indication of a lowered activity. The members of the *Drosophila pseudoobscura* group exhibit greater activity by a higher oxygen consumption and by a higher frequency of wing beats. Observable differences in activity are also evident to the laboratory observer. The greater fecundity of the one race (A) at a higher temperature than of another form (B) may be an indication of activity differences at the temperatures compared. Motion or flight activity in captivity in other groups has been observed directly.

TABLE VIII

THE INFORMATION AVAILABLE IN THE SPECIES GROUPS AND THE DEGREE OF THEIR FIT IN THE CORRELATIONS

Key: +, information available and a fit with the variation expected.
Blank, no information available.
±, information available, data in agreement, but uncertain, or not directly applicable.
+*, a partial disagreement with the direction of variation expected in the group, but the general trend being in agreement.
+°, an apparent disagreement, due to different pigment involved.
0, information available, but no positive or negative fit.

	Culex pipiens	*Anopheles maculipennis*	*Anopheles albitarsus*	*Haemagogus*	*Drosophila* species	*Drosophila pseudoobscura*	*Colias*	Other Lepidoptera
A	±			+	+	+	±	+
B	±			+		+	+	+
C	+	+*		+			+	+
D	+	+	+	+	+*	+	+	+
E	+*	+	+	+	+	+	+°	+
F	+			±				
G	+	+						
H	+	+	+					
I	+*	+		+*		+*	0	0

The members of many groups of insects differ in their ability to mate in a small enclosed space. Some forms require very little space for flight. Others require a large area before the sexes will be attracted or will copulate. In each group a series of forms requiring from little space to large space may be arranged. In each of the groups, *Culex pipiens, Anopheles maculipennis, Haemagogus, Colias,* and *Melitaea,* such a series may be arranged. Each of these falls into concomitant association with the other physiological characters, namely, rate of development and activity. There is, however, a discordant species in the *Anopheles maculipennis* group concerning mating habits.

The ecological distributions of the members of these species-groups differ in that some forms exist in a cold or damp climate and others in a hot and dry one. Each group (Table VIII) forms parallel series with the other groups in regard to this character. Species which prefer cold and damp climates are sluggish (less active), have a slower development rate, and need a large space for mating. Owing to the fact that the relationships hold well only for closely related members of a group, the general species of the genus *Drosophila* possess this relationship in a statistical sense only. Others, such as the *Anopheles maculipennis* group and *Haemagogus* show it best when subspecies rather than species are compared. The greater the phylogenetic diversity the less the amount of regularity which can be expected in direct comparisons.

In the darkness of melanin pigmentation or of chitin coloring, the members of the groups fall into series which vary concomitantly with other characters. Darkness of pigmentation is associated with a cold and damp climate, a sluggish behavior, a slow development rate, and the necessity for a large space for mating. In *Colias,* a pigment of a different chemical nature from melanin gives a reversal to the usual associations for color. With respect to melanin in this genus there is no reversal. In *Culex pipiens,* there is a partial lightening of color where a darkening might be expected, but the general body is dark, as expected.

The relationships of the tolerance to various concentrations of larval food and salt solution have been determined in only three groups (only two thoroughly. In *Culex pipiens,* various concentrations of salt solutions and of density of micro-organism infusions have been tested. In *Anopheles maculipennis* only salt solutions and in *Haemagogus* only infusions have been tested. In all three cases the forms in a group which can withstand the most concentrated solutions and infusions are those which have the fastest development rates, have the greater activity, can mate in a small space, prefer hot or dry environments, and are pale.

The characteristic concerned with the adult feeding habits can be applied to mosquitoes only. For a blood meal these have a choice between man and animals. In each of the three groups on which information is available, namely, *Culex pipiens, Anopheles maculipennis,* and *albitarsus,* the species which bite man are those which also have the characteristics enumerated in the preceding paragraph (see also Table VIII).

Associated with these physiological characteristics are certain morphological ones which have been discovered to aid in the differentiation of closely related species. They are generally body-size characters or differences in specific proportions and amount of development of body protuberances or appendages. In *Culex pipiens,* these characters are: number of branches to the hairs, hair length on the larvae, palpi length in relation to the proboscis, relative length of the siphon, and the number of branches in the siphonal tufts of the larvae. In relation to each of these characteristics one form might be described as having fewer or being shorter or smaller, and the other as having more or being longer or larger. Thus, all characteristics vary in parallel, except the number of branches in the siphonal tufts, which is greater when the other characters are on the decrease. In compensation for this exception, the length of these tufts is shorter when the number of branches increases.

The morphological characters which are considered in *Anopheles maculipennis* are the number of branches of antepalmate hairs in the larvae, the relative length of the wings to the body, the robustness of some spines on the male genitalia, and the expansion of hairs on the second segment of the larvae. In all instances, when the group is divided into three natural parts, there is a correlation between increased number of hairs, length of parts, robustness of parts, and expansion of hairs.

The morphological characters of the *Haemagogus* mosquitoes fall into the same general classes. Four different sets of hairs have been counted. The number of scales in the comb and pecten and in the pecten tuft of the male genitalia and the ratio of the siphon length to its width have been analyzed. With few exceptions, when the number of hairs or setae increases at the different loci (seven loci), the number of scales increases at the other loci (two loci) and the appendage length increases (siphon length). In the three cases in which exceptions have been encountered, the exceptions have been compensated for by increases in other ways, that is, if the numbers of hairs or scales have decreased where an increase is expected, an increase in length may compensate (Table V).

Only three morphological characters are analyzed in *Drosophila pseudoobscura,* namely, number of sex-comb teeth and the size of the wing and tibia. As these measurements were not comparable with some part of the body, they are difficult to interpret. The variation of all characters is parallel in these three forms (Table VII).

In *Culex pipiens, Anopheles maculipennis,* or *Haemagogus* a corresponding type of morphological variation has been shown to be associated with corresponding types of physiological variations. The increase in the numbers of hairs or scales or in length of body appendages or hairs is correlated with a slower development rate, a sluggishness of the adults, a requirement of greater space for the copulation flight, a preference for cooler and wetter environments, a darker melanin coloration, a poor larval food and salt tolerance, and an adult preference for animal blood. Races A and B of *Drosophila pseudoobscura* exhibit an indirect relationship which is difficult to explain, since *D. miranda* shows a direct one. But this may be explained below.

Interpretation

The interpretation of these phenomena must be based upon biological or natural processes at two levels. The first level is marked by the association of different characters in a species group of animals and their concomitant variation within that group. At the second level is the repeated variation or parallel association of these characters in discrete species groups. With an explanation secured for the first level, the second will readily be understood.

An investigation into the developmental mechanics of the characters discussed above may throw some light on the meaning of their association. One is handicapped here, however, since the information in these fields is very meager. Most of the fundamental principles governing the developmental physiology of the characters under discussion are still unknown. Without a knowledge of the interrelationships in development of these various characteristics, most hypotheses will fall far short of their goal.

The associations of the characters described above and their concomitant variation within a species group might be due to one or other or both of two genetic mechanisms. First, all the characters may be controlled in development by the same gene or by a closely linked group of genes. This would be an example of the pleiotropic or manifold effects of genes for which increasing evidence is becoming available. It would seem illogical to attribute all the associations between the characters of these animals, however, to such a mechanism. The second possible interpretation is independent of the first. Each character might be independently regulated in development and happen to be associated because of selection by the same factors of the environment. The evidence indicates that each of these possible mechanisms probably plays some part in the development of the associations described.

Some of the best evidence supporting the pleiotropic nature of many of the associations has been summarized by Neel (1940) from other authors, all pertinent parts being reproduced in Table IX. This table shows the correlation between character expression and increased temperature and increased body size in *Drosophila melanogaster* mutants and wild type. The primary effect of temperature on the body processes in insects is to increase or decrease the body activity. An indirect relation may then be made between

TABLE IX

A Comparison of the Correlation Between Character Expression and Higher Temperature and Greater Body Size (*D. melanogaster*)

Character	Higher Temperature	Greater Size
Wild-type wing length	Decreased	Increased
Fore-femur length	Decreased	Increased
Number of teeth in wild-type sex comb	Increased	Increased
Vestigial wings	Decreased	Increased
Scute bristles	Some increase Some decrease	Same

increased activity and character expression. The wild-type wing length was decreased by higher temperature, but also increased with increase in body size. The fore-femur length was decreased by higher temperature and increased by larger body size. The number of teeth in the wild-type sex comb was increased with a higher temperature and also increased with larger body size. The size of vestigial wings was decreased by increased temperature and increased with increased body size. The number of scute bristles was increased in the case of some strains and decreased in others.

Comparison of these results with the associations of characters in wild forms described in this paper shows significant correlations. *Drosophila pseudoobscura* (Table VII) shows an increase in number of teeth in the sex combs in relation to the size of the form; that is, race B is the smallest and has the smallest number of teeth, race A is intermediate, and *D. miranda,* the largest in size, has the greatest number of teeth. Tibia length, wing size, and number of teeth in the sex combs also are directly correlated. Since the correlation of these characters is probably independent of some of the other characters, the misfit of these data as indicated in Table VIII is not illogical. As the sex comb teeth number is also correlated with increased temperature (increased activity), race A is not out of place in having a larger number of teeth than race B; *miranda,* however, is. One cannot expect the characters to fit two opposed factors.

The relation of activity and temperature to bristle number increase or decrease is variable. Nevertheless, in most stocks there were more bristles induced at lower temperatures and fewer at the higher temperatures (Child, 1936). There is a direct correlation between the size of the body and the number of chaetae at various places on the body (Neel, 1940 and 1943), the larger the size, the more bristles at any locus. Body size is indirectly correlated with temperature in *Drosophila;* large flies are produced at lower temperatures and smaller ones at higher (Stanley, 1935; Combs, 1937). Thus, at the lower temperatures more bristles should be produced. Ives (1939) found more bristles in a scute mutant at high temperatures and less in a wild type. But it is a wild type with which we are concerned.

A comparison of these correlated developmental phenomena shows that the forms which exhibit a more rapid development rate and greater activity are also those forms which have fewer parts in or a lesser development of the hairs, appendages, etc. (*Culex pipiens, Anopheles maculipennis, Haemagogus, Drosophila pseudoobscura*). In most of the forms with an increase in bristle, hair, or chaetae number or length, there is a parallel variation of these units in all parts of the body. This is present despite the fact that different loci are under different genetic regulation as judged by their independent variations (Ives, 1939; Helfer, 1939; and Neel, 1941). There is however, in polychaetoid *D. melanogaster,* a relation between the number of bristles present at a body locus and their total length (Neel, 1940).

There are numerous instances, reported even in old literature, in which increased temperature and activity result in a paling of the colors of the body. This relationship holds generally for chitin tints and melanin deposition. A specific instance has been studied in *Colias* (Hovanitz, 1946*b*), in which another pigment (pterine) behaves in an inverse fashion from that of melanin even though at the same time melanin varies according to rule. It would be an oversimplification and would not basically solve the problem to suggest that metabolism and color of the pigment may be directly related to general body activity. General activity may be increased either by a temperature increase or by a genetic mechanism. The result in either case would be physiologically comparable, leading to an organism having all properties adjusted to a more rapid rate of development.

The correlation of these characters with the natural environmental conditions follows the basic developmental pattern.. Those species existing at the higher temperatures have the characteristics expected of forms with greater metabolic activity, and those which live at the lower temperatures have the characteristics expected of forms with lower metabolic activity. There is nothing strange about these relationships. Nevertheless, but little seems to be known as yet of the fundamental principles of development by which these relationships are produced. Until it can be shown definitely that there is a selection for the forms which possess a higher metabolic level in the ecological areas where such a level is advantageous, such a deduction must remain unproved. The Lamarkian hypotheses need not be invoked; neither, on the other extreme, need the hypotheses of some yet unknown character be postulated.

Associations of characters of these sorts are not restricted to insects. Similar relationships have been pointed out in various laws of geographical variation in vertebrates. Apparently, pleiotropic effects are not uncommon for certain of the genes that regulate the characters of color, activity, and size of body parts of animals. The pleiotropic effects are not yet demonstrated beyond question in many cases, but they may be looked for now that their existence is expected. In mice, MacArthur (1944), and in rats, Keeler (1942), Keeler and King (1942), and Shopbach, Keeler, and Greenberg (1943) have shown that some genes may have pleiotropic effects. Correlations of these genetic characters with the environments of the animals have not been made. Field work has shown color and size characters in correlation with the environment. Laboratory work indicates a developmental relation between activity, color, and body proportions. A more direct correlation between the three latter characters and the environment is to be looked for.

From the standpoint of medical entomology it is interesting to note that it is those species of mosquitoes which have characteristics correlated with greater activity which are the pests of man and the carriers of disease. For example, the form *molestus* in the *Culex pipiens* group is the species which inhabits houses and prefers to bite man rather than animals. Several of its characteristics make it well able to do this. A more rapid development rate is advantageous in a temporary pool where the larvae are breeding. The ability to mate in small containers is advantageous, when only such small containers are available in houses and barns. The greater activity of the adults and their preference for a blood meal from man are advantageous in a rapidly changing environment of this sort.

Anopheles maculipennis forms of this type are also those which bite man and which transmit malaria in the regions in which they exist. These are *elutus, labranchiae* and *atroparvus.* The *Anopheles albitarsus* form having corresponding characteristics is also a pest to man and the carrier of malaria in its region.

The evidence presented suggests that the parallel association of distinct characters in these discrete groups of organisms may be due to one or both of two genetic mechanisms. First, though the characters may be distinct in form they may be connected in development by a pleiotropic or manifold effect of the same developmental processes so that when one character varies the other also does. Second, several characters may be selected by the same environmental factors, leading to a corresponding association in the same organism.

SUMMARY

1. The association of several characters has been shown to occur in discrete species groups of mosquitoes, *Drosophila,* and Lepidoptera. These characters vary in a parallel fashion among the members of the groups.

2. The characters shown to be associated are various qualities of (A) development rate, (B) activity of adults, (C) mating habits, (D) ecological distribution, (E) coloration, (F) larval food tolerance, (G) salt tolerance, (H) adult feeding preference, and (I) morphological characteristics. The physiological characters mentioned (A–H) seem to be related to speed of body metabolism. The morphological characteristics (I) are concerned with body proportions and extent of manifestation of hair, chaetae, and spine characters.

3. The parallel variation exhibited by the several taxonomic groups is of the following sort: if the development rate is rapid, the adult activity is great, the adults will mate in small space, the insect prefers a hotter and dryer environment in which to live, its colors are lighter, the larval food and salt solution tolerances are greater, the adults (in mosquitoes) will feed on man, and there is an increase in extent of manifestation of the particular morphological characters concerned.

4. Many of the associations and parallel variations seem to be pleiotropic (manifold) effects in development. The genes concerned are probably metabolic rate genes, each having pleiotropic effects and together having an additive influence on each character. It appears probable that one or more of the associated characters are advantageous to a given form in its specific environment, though no direct evidence of selection is now available for most

of these characters. The other associated characters then may either be evolved in a parallel fashion due to parallel selection or they may be pleiotropic effects.

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